Toxicity of the Herbicides 2,4-D and Glyphosate to Newfoundland Brook Trout (*Salvelinus fontinalis*) and Atlantic Salmon (*Salmo salar*)

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July 1987

TABLE OF CONTENTS

ACKNOWLEDGEMENTS..... iii

INTRODUCTION..... 1

MATERIALS AND METHODS..... 2

Field Methods..... 2

Fish Holding Techniques..... 5

Acclimation to Experimental Temperatures..... 6

Laboratory Methods..... 7

Preparation of Test Containers..... 7

Preparation of Toxicant Mixtures..... 8

Monitoring of Toxicity Tests..... 9

Chemical Analysis..... 10

Glyphosate..... 10

2,4-D..... 10

Toxicant Concentrations and Correction Factors..... 10

Calculation of LC50..... 14

RESULTS AND DISCUSSION..... 16

Characteristics of Test Specimens..... 16

Environmental Conditions..... 16

Reference Toxicant..... 19

Toxicity of 2,4-D Formulations..... 19

Toxicity of Glyphosate Formulation..... 20

Comparison with Other Studies..... 20

CONCLUSIONS.....25

Study Results.....25

Application of Results.....25

LITERATURE CITED.....28

APPENDIX 1.....30

ACKNOWLEDGEMENTS

This study was funded by The Newfoundland and Labrador Department of Forest Resources and Lands, Abitibi-Price, and Corner Brook Pulp and Paper Limited. George Ross, Bill Furey, and George Van Dusen of those organizations, respectively, are gratefully acknowledged for their role in coordinating the administration of the project.

The Environmental Protection Service (EPS), Environment Canada, St. John's, provided the toxicity laboratory facilities, without which it is unlikely that the study could have been completed within the required time frame. Jim Osborne and Bruce Moores of EPS kindly expedited the use of these facilities. Richard Martin and Coleen Leeder of EPS provided useful advice throughout the laboratory portion of the study.

Bob Wiseman, John Gibson and Rex Porter of the Department of Fisheries and Oceans (DFO) provided helpful information on the field collection part of the project. Rick McCubbin of DFO provided advice on the final report. Malcolm Foy , consultant to LGL, provided valuable advice on study design and also reviewed the report. Chris Holdsworth of LGL assisted in data analysis.

Chemical analysis of herbicide solutions was provided by Dr. Ross McCurdy of the Victoria General Hospital Environmental Chemistry Lab, Halifax. Analysis of copper solutions was provided by the Memorial University Water Analysis Facility.

INTRODUCTION

The present study was conducted for the Newfoundland and Labrador Department of Forest Resources and Lands, Abitibi-Price, and Corner Brook Pulp and Paper Limited. It is intended to fill a data gap identified during the environmental review stage of the proposed Herbicide Application Program. The program has been proposed to aid in the silviculture of valuable softwoods in the province.

It was identified during the review process that no information was available on the toxicity of the proposed herbicides to local populations of young brook trout (Salvelinus fontinalis) and Atlantic salmon (Salmo salar). Thus, the present study was instituted to conduct toxicity tests using wild specimens of these populations and the appropriate herbicide formulations and temperatures. The resulting information will aid in the assessment of impact of the proposed spraying program.

Acute lethal toxicity tests (static) were conducted on brook trout at 15°C using dy-amine and iso-octyl ester formulations of 2,4-D (2,4-dichlorophenoxyacetic acid), and a glyphosate (N-(phosphonomethyl) glycine) formulation. Atlantic salmon, primarily the landlocked form, known locally as ouananiche, were tested with the amine and glyphosate formulations at 15°C and the ester formulation at 10° and 20°C. 96hLC50's and 95% confidence limits were calculated and presented.

MATERIALS AND METHODS

Test specimen collection and holding methods, and laboratory and data analysis techniques are detailed in the following sections. Where possible, general toxicity testing methods as outlined in Sprague (1973) and EPS (1982) were utilized.

Field Methods

Test specimens of juvenile brook trout and Atlantic salmon (primarily ouananiche, with the possibility of a few sea-run ones from the North Arm River drainage) were collected from a variety of streams on the Avalon Peninsula during October 1986 (Figure 1; Table 1). Water quality data for the collection sites are given in Table 1.

Fish were collected by electrofishing and dipnet and immediately placed in plastic buckets lined with green polyethylene bags and held in stream water at ambient temperature. Never more than 200 specimens were collected per trip and these were transferred to large plastic barrels, lined with polyethylene bags, for transport to the EPS laboratory in St. John's. In most cases, no more than five hours passed between collection and placement into the EPS holding tanks. Mortality due to stress from temperature shock or low oxygen levels was virtually non-existent. Total number of direct fatalities in the field was approximately ten fish and these appeared to be caused by mechanical injury. While it is recognized that electrofishing may not be the best collection method for toxicity tests since fish may undergo some physiological stress (Rand and Petrocelli 1985), it is the only practical method available for collecting large numbers of juvenile salmonids under Newfoundland conditions. Beach seining for small juveniles would be ineffective due to their behavioural trait of fleeing into the substrate when alarmed. The substrate in all cases was composed of a high percentage of boulders and cobble. Trapping might be possible but would be very time-consuming. To alleviate some of the concern about physiological stress due to collection method, a relatively long acclimation period was used prior to conducting the toxicity tests.

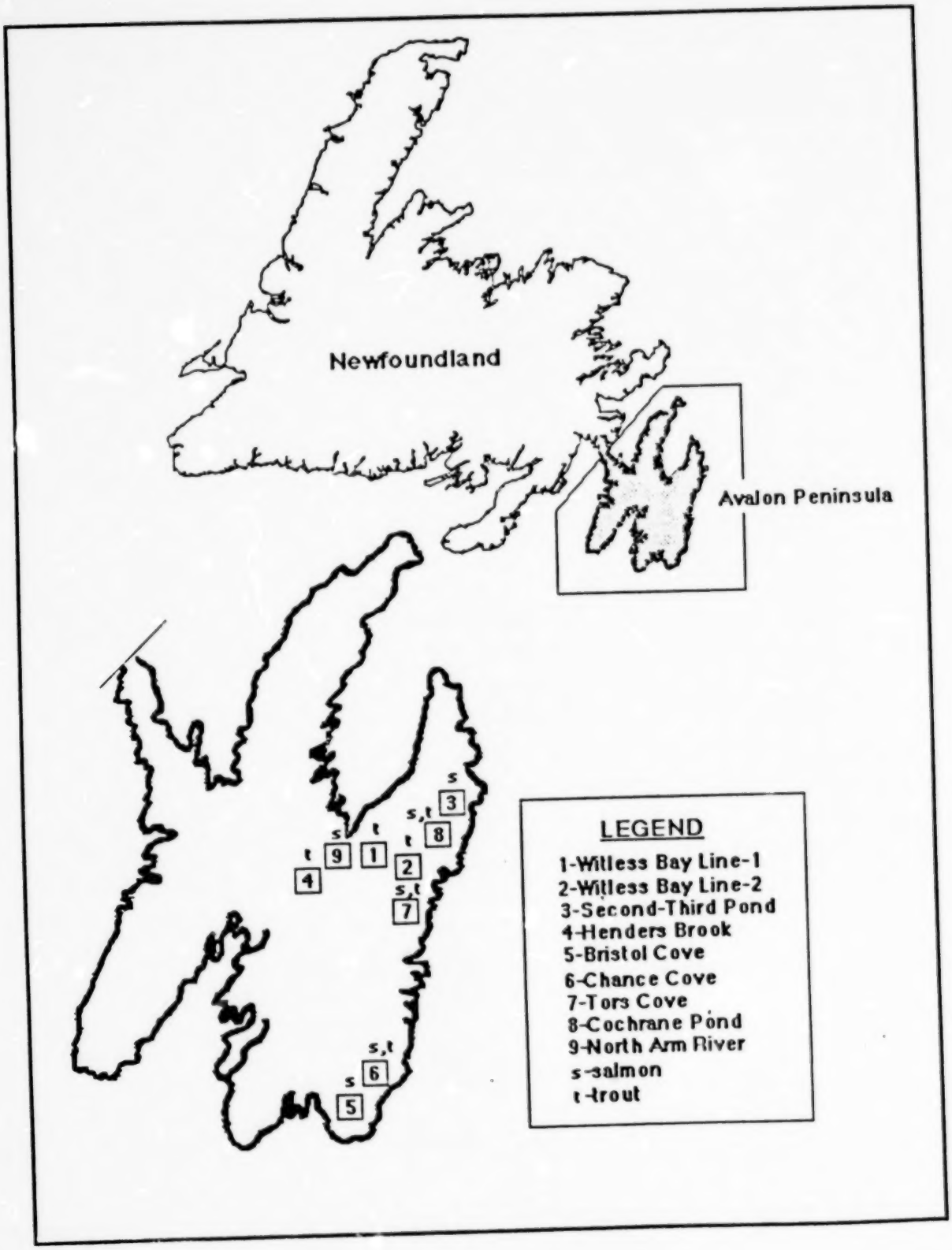


Figure 1. Locations of collection of test specimens.

Table 1. Collection locations of test specimens, 3 October to 21 October 1986.

Location	Date	Species	Approx. No.	T(°C)	Hardness (mg/L)	pH
WBL-1	3/10	BT	42	9	6	6.5
	7/10	BT	76	-	-	-
	21/10	BT	18	-	-	-
WBL-2	21/10	BT	36	6	7	5.9
2nd/3rd Pond	3/10	S	62	10	9	6.6
Henders Brook	7/10	BT	50	8	7	-
	21/10	BT	21	7	-	-
Bristol Cove	8/10	S	10	10	7	6.4
Chance Cove	8/10	S	74	9	6	6.4
		BT	10	-	-	-
	11/10	S	75	7	-	-
Tors Cove	9/10	S	107	10	8	-
		BT	43	-	-	-
	11/10	S	93	8	-	-
		BT	22	-	-	-
Cochrane Pond	14/10	S	21	8	6	-
		BT	24	-	-	-
North Arm R.	16/10	S	49	10	9	6.5
TOTAL NO.		S	491			
		BT	342			

Abbreviations: WBL=Witless Bay Line; S=salmon; BT=brook trout.

Fish Holding Techniques

Fish were held in a large EPS holding tank ($0.34m^3$) at ambient conditions for varying periods (1 to 21 days) from 3-24 October 1986 until transfer to the temperature acclimation tanks. Water flow was continuous via the flow-through, sterilized (u.v.) charcoal-filtered system (Culligan filters) utilized by the EPS facility. Water originates from the St. John's water supply. Alkalinity of holding water was 2.6 mg/L $CaCo_3$; hardness was 8.7 mg/L $CaCO_3$ (Ca^{++}=2.31 mg/L; Mg^{++}=0.71 mg/L), and pH was 6.51. (Determinations of alkalinity, hardness, and pH were made by Memorial University Water Analysis Facility according to methods outlined in APHA 1975.) Lighting regime in the aquarium room was normally about 12h of fluorescent light and 12h of darkness.

Temperature, tank and fish condition were monitored at least once daily and fish were fed slowly over a twenty minute period once daily. Initially fish were fed a wide variety of foods, including chopped beef liver, in an attempt to induce them to feed vigorously. Once feeding, the primary food item was freeze-dried marine zooplankton (Murex brand; medium size).

Some mortality was observed in the holding tanks. Total mortality during the entire holding and acclimation periods was approximately 43% for trout and 29% for salmon. In the case of salmon this appeared to be due primarily to collection stress since virtually all mortalities occurred at the beginning of the holding period. An uncounted number of fish, primarily salmon, were also lost when they jumped out of the holding tanks. Trout mortalities occurred in low numbers throughout the holding period and these specimens appeared to be thinner than normal. However, many trout were observed feeding aggressively and not all of the trout were characterized by low body weights.

It is unknown if the total mortality observed during holding was high relative to what the mortality is in the 'wild' since the natural mortality rate for these populations is unknown. However, it is clear that test specimens were healthy since total control mortrality was less than 10% and the fish were relatively resistant to the reference toxicant.

Total mortality was less than 10% during temperature acclimation periods and, in general, test specimens appeared healthy and were feeding

aggressively prior to testing.

Acclimation to Experimental Temperatures

Fish collections and general holding of specimens occurred from 3 October to 24 October 1986. During that period all fish were placed in the large holding tank that was held at the ambient temperature of the EPS flow-through system. Temperatures during this period varied on a daily basis but were normally about 12-15°C. Thus, all fish were acclimated to general laboratory conditions from 1 to 21 days before the temperature acclimation periods began. After the last collection trip, brook trout and salmon for the 15°C tests were acclimated in a large circular tank (0.34m^3) heated by two 850 watt submersible heaters. Salmon for 10° and 20°C tests were acclimated in smaller rectangular tanks (0.17m^3); heat was supplied by two 100 watt aquarium heaters in the former and two 850 watt heaters in the latter. Water in the large tank was brought up to 15°C over several days and some of the salmon were separated into smaller tanks for eventual acclimation for the 10°C and 20°C experiments. Attempts were made to randomize allocation of fish to specific tanks by alternately placing one or two fish in one tank and then one or two in another tank. Actual acclimation times at specific temperatures are shown below.

Species	Temperature °C	Temperature Acclimation (d)	Total Acclimation (d)
Brook trout	15	19	22-40
Salmon	15	23	30-44
Salmon	10	23	30-44
Salmon	20	12	19-33

Temperature measurements in the 15°C tank ranged from 13.0 to 16.2°C but were generally about 14.6°C. Temperature measurements in the 10°C tank ranged from 8.0 to 9.0°C. Temperature measurements in the 20°C tank were more variable than the other tanks due to the large difference in temperature between the water entering the system and the desired

temperature; they ranged from 15.0°C (on one day) to 20.0°C with most temperature measurements between 18 and 20°C.

Salmon for the 20°C experiment were not subjected to a full 21 day temperature acclimation period as suggested by EPS (1982). The temperature acclimation period was shortened since the experiments were running behind schedule due to initial delays in obtaining specimens, equipment in the 20°C acclimation tank was not performing well, and there was concern that if we had accidental mortalities we would be unable to replace the fish due to the lateness of the season. Thus, it was decided to shorten the time at temperature to 12 days rather than risk a complete loss of data. In our opinion, this decision did not invalidate the data since the fish were acclimated to laboratory conditions for 19-33 days and the final results appeared to be consistent with the other experiments. In addition, it can be argued that temperature acclimation for experiments with wild specimens may be irrelevant since they are subject to wide daily fluctuations in temperature in their natural environment. Acclimation periods listed in the literature for similar experiments range from 0 to 21 days or more.

Laboratory Methods

In general, each experiment consisted of five test containers containing five different concentrations of herbicide mixture, two control containers, and one set of containers with three different concentrations of reference toxicant. Each container held between 7 and 11 fish depending upon the experiment. Containers were not aerated during the course of the experiments. Tests were 'static' and toxicant mixtures were not changed throughout the 96h test period. Great care was taken to prevent cross-contamination of the test containers and all glassware and implements were carefully washed and rinsed after each use.

All toxicity testing was conducted in the EPS toxicity laboratory in St. John's . Detailed methods are described below.

Preparation of Test Containers

Test containers ($0.05 m^3$), positioned in the waterbaths, were prepared

24h prior to the experiments by lining with heavy polyethylene bags (clear) and by adding 45L of filtered system water. Containers were then aerated for 24h using the Lab's on-tap air supply and air stones. Temperature of the waterbath was maintained by a recirculating heating/cooling unit that serviced the waterbath. After 24h, toxicants, and then fish, were added. Position in the waterbath of each container/mixture was randomized using a random number generator. Fish were evenly distributed among the test containers by adding one fish to each tank in series and then repeating the sequence until the desired number of fish was added. All experiments allowed at least 0.5 L of test solution per gram of fish per 24h period of exposure as suggested in EPS (1982).

Preparation of Toxicant Mixtures

Sealed, new containers of the three commercial herbicide formulations were received from the Newfoundland Department of Forest Resources and Lands. Specific formulations were Pfizer dy-Amine (active ingredient 2,4-D alkanolamine salts, d-isomer 470g/L) and Pfizer 2,4-D Ester L.V. 600 (active ingredient 2,4-D iso-octyl esters, d-isomer 564g/L), and Monsanto Roundup (active ingredient glyphosate, isopropylamine salt 356g/L). Fisher Chemical cupric chloride ($CuCl_2*2H_2O$; crystal; purified) was utilized as a reference toxicant. Nominal concentrations were prepared based upon the concentration of active ingredient (total ingredient in the case of cupric chloride) in the formulation by transferring the toxicant (or diluted toxicant in the case of glyphosate and cupric chloride where dilute solutions were made up using distilled water and stirred with magnetic stirrer prior to use) to the water-filled test containers by volumetric pipet. Mixtures were stirred by glass rod for one minute each. All toxicants seemed to readily mix with water. However, the ester formulation created a cloudy mixture that was maintained throughout the test period; it left a white residue on the bottom of the containers after 96h. According to the handling instructions (Pfizer handling label; Registration No. 9561 with Pest Control Products Act), this formulation forms an emulsion in water rather than a solution. Removable plastic mesh liners were used in some of the ester formulation experiments since the cloudy condition of the water precluded accurate observations of mortality. In these cases, liners were elevated in the test container at observation time in order to bring the fish near the surface where they could be observed.

Monitoring of Toxicity Tests

Tests were monitored for at least four intervals within 24h, and then 48h, 72h, and 96h to determine mortality at those time intervals. Death was defined as the total lack of movement after stimulus such as prodding with a glass rod and was usually characterized by rigor mortis, and large mouth and gill gapes. Dead fish were removed from the test containers by small stainless steel strainer attached to a glass rod or by teflon strainer attached to a hardwood rod. Dead specimens were identified to species, measured by calipers to the nearest m m and weighed to the nearest 0.1g by electronic balance and preserved in 5% formalin.

Temperature in the test containers was monitored by mercury thermometer (+/-0.1°C) at 24h, 48h, 72h, and 96h. Oxygen was measured in one or two containers per observation period by modified Winkler procedure (Strickland and Parsons 1972) or Hach Kit; similarly, pH was spot-checked by Accumet pH Meter Model 640A (+/- 0.02 units). These variables were monitored since they may influence toxicity.

Samples of reference toxicant solutions were collected at the beginning and end of every experiment by dipping a new 250 m L polyethylene bottle into the test mixture. Samples were preserved with 1.0 m L of analytical grade nitric acid. Analysis for low level copper (total copper) was conducted by Memorial University Water Analysis Facility using the carbon rod flameless atomic absorption technique (APHA 1975).

All herbicide mixtures for Experiments 1 and 2 (brook trout; 15°C) were sampled at the beginning and end of the experiments. Thereafter, sampling was confined to ester (60ppm nominal) since it appeared that this formulation was not particularly soluble in water and thus was most likely to change significantly during 96h. It was assumed that the chemical behaviour of all of the mixtures would be similar at 10° and 20°C compared to behaviour at 15°C. Herbicide samples were collected in washed,, amber glass, narrow neck 250 m L bottles that were double-rinsed with distilled water and then acetone. The caps were sealed with aluminum foil and the samples stored in a refrigerator prior to analysis.

Chemical Analysis

Sample analysis was conducted by Dr. Ross McCurdy of the Victoria General Hospital Environmental Chemistry Lab, Halifax, using gas chromatography. Methods are detailed below (McCurdy, pers. comm.).

Glyphosate

Glyphosate [N-(phosphonomethyl) glycine] in water was concentrated by thin-film rotary evaporation, purified through AG 50W-X8 (H^+), eluted with distilled-deionized water, and concentrated to near dryness by rotary evaporation and to dryness under dry nitrogen. The residue was dissolved in trifluoroacetic acid, refluxed with trifluoroacetic anhydride, and evaporated to dryness under dry nitrogen. The residue was dissolved in methanol and methylated with diazo-methane, brought to near dryness under dry nitrogen and heat and quantitatively diluted to 5.0 mL with tetrahydrofuran. The glyphosate derivative was identified by GC-MS and GC-EC using a DB-5, 30 meter capillary column.

2,4-D

2,4-D (2,4-dichloro phenoxy acetic acid) and its esters and amine salts were extracted from water with three separate chloroform extractions. The extracts were washed with distilled-deionized water and the chloroform was passed through an acidified activated sodium sulfate column, concentrated to 1.0 mL by thin-film rotary evaporation, evaporated to dryness under dry nitrogen, methylated with diazo-methane, evaporated to dryness under dry nitrogen and quantitatively diluted to 5.0 mL with hexane. The resultant methylated forms of 2,4-D are identified and quantified by GC-EC using a 30 meter DB-5 capillary column.

Toxicant Concentrations and Correction Factors

Nominal and measured concentrations of the various toxicant mixtures based upon active ingredients are shown in Table 2. Nominal

Table 2. Nominal and measured concentrations of toxicant mixtures. Cu is ppb; other toxicants are in ppm.

Experiment	Toxicant	Nominal Conc. Active Ingred. (ppm/ppb)	Nominal Conc. Formulation (mL/L)	Measured (ppm/ppb) 0h	96h	Average
1	Amine	30	0.06	30	37	34
	Amine	54	0.11	57	56	57
	Amine	96	0.19	97	97	97
	Amine	168	0.34	176	158	167
	Amine	250	0.50	212	235	224
	Glyphosate	3.0	0.01	0.9	1.0	1.0
	Glyphosate	5.4	0.02	1.7	1.4	1.6
	Glyphosate	9.6	0.03	3.9	3.7	3.8
	Glyphosate	16.9	0.05	4.3	4.2	4.3
	Glyphosate	30.0	0.09	9.6	9.5	9.6
	Cu	5	-	<2	3	2
	Cu	20	-	<2	4	3
	Cu	50	-	13	14	14
2	Ester	30	0.06	34	24	29
	Ester	54	0.11	33	29	31
	Ester	96	0.19	56	48	52
	Ester	168	0.34	47	56	52
	Ester	250	0.50	77	133[1]	105
	Cu	5	-	<2	<2	<2
	Cu	20	-	4	6	5
		50	-	13	17	15
3	Cu	50	-	13	16	15
	Cu	100	-	31	37	34
	Cu	200	-	61	65	63
4	Ester	Ester 60	0.12	23	36	30
	Cu	50	-	13	21	17
	Cu	100	-	35	35	35
	Cu	200	-	59	64	62
5	Ester	Ester 60	0.12	25	67	46
	Cu	50	-	17	18	18
	Cu	100	-	28	55(28)	42(28)[2]
	Cu	200	-	55	59	57

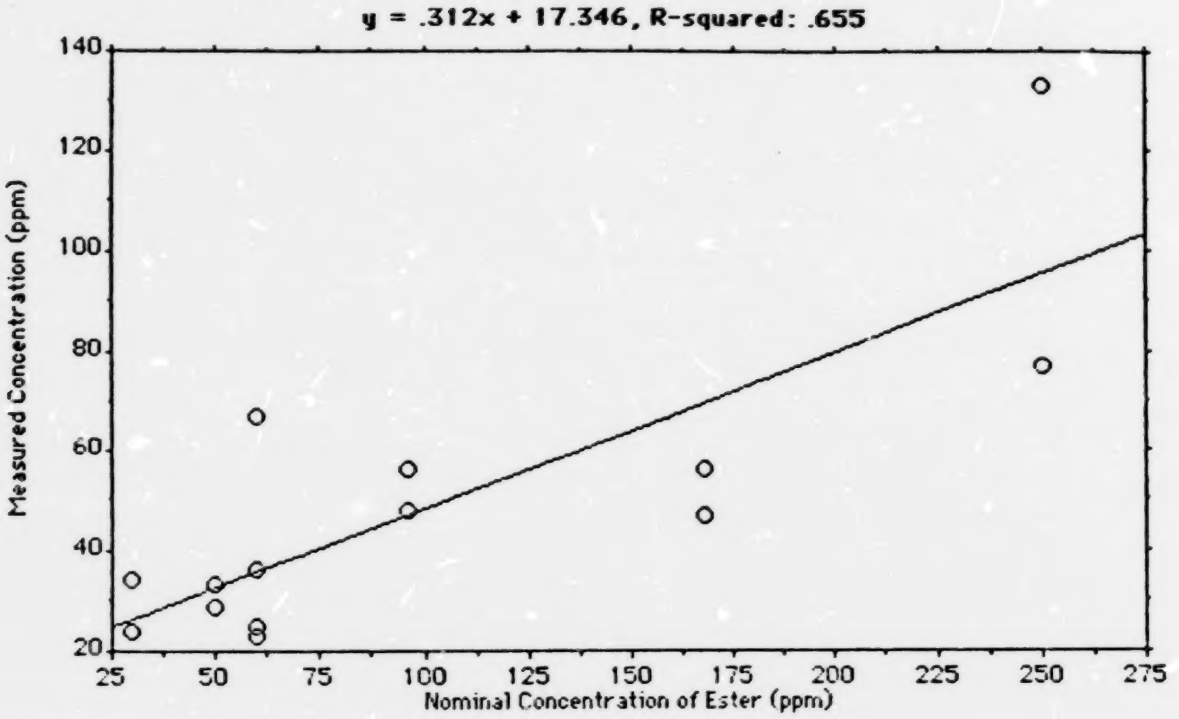
[1] This value seems exceptionally high; reason is unknown but may be related to the behaviour of this formulation in water (e.g. sample may have contained some of the white precipitate that may be high in ester).

[2] 28ppb was used as the value most likely to be correct.

concentrations of total formulation are also shown for comparison purposes. It should be noted that the measured values refer only to the active ingredient of the respective herbicide formulation and are expressed as ppm (i.e. mg/L). Cupric chloride ($CuCl_2*2H_2O$) concentrations refer to measured copper concentrations (ppb total copper). The copper values used in calculating the LC50's for each experiment were those obtained by averaging the 0h and 96h measured concentrations for those particular experiments. Copper concentrations did not appear to change during the course of the experiments. The 0h value was used for the 20°C ester experiment (Experiment 5) since the 96h value was probably an error.

Measured concentrations of the amine formulation were very similar to the nominal concentrations (as based upon the amount of toxicant added) and no correction factor was used in the calculation of the LC50 (Table 2). Measured values of glyphosate were consistently lower than the nominal concentrations and, on average, were 0.32 (+/-0.05) the nominal concentrations (Table 2). A correction factor (0.31) was used in calculating the final LC50 for the glyphosate formulation. This factor ($y=0.306x+0.042$; $R^2=0.962$; $p<0.05$) was obtained by regressing measured values against nominal values (Figure 2). Measured values of the ester formulation appear to be indicative of the nominal values at the low end of the ranges used (i.e. 30ppm) but are considerably different at the higher end of the range (i.e.>96ppm). Similar to the glyphosate procedures, we developed a correction factor by regressing nominal concentrations against measured concentrations of ester to obtain the formula: $y=0.312x+17.346$; $R^2=0.655$; $p<0.05$ (Figure 2).

The specific reasons for the observed differences between added and measured concentrations of two of the formulations (glyphosate and ester) are not known at present but are undoubtedly related to the chemical and physical behaviour of these formulations in water and to the analysis techniques used. The ester, in particular, may create problems for adequate sampling, and thus for chemical analysis, since it lacks high solubility in water. The ester formulation, particularly at the higher concentrations utilized, created cloudy test solutions that left a white residue in the containers after the water was removed at the end of each test.



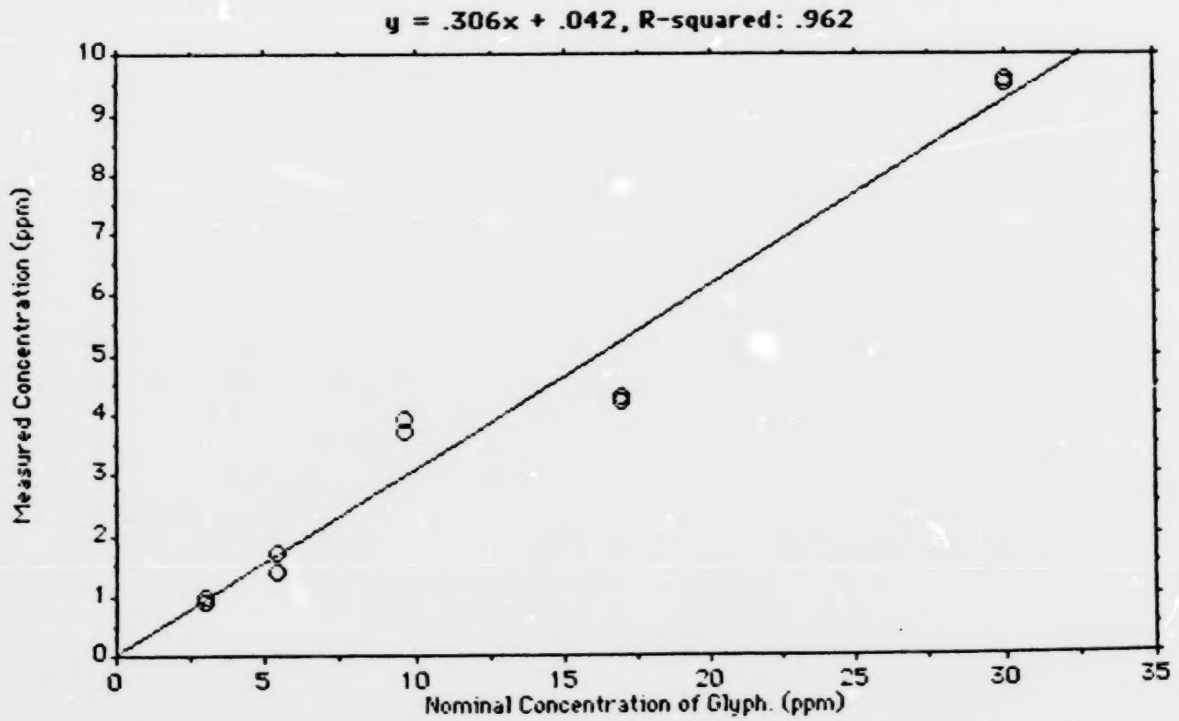


Figure 2. Regression of nominal ester and glyphosate against measured concentrations.

Calculation of LC50

There are a variety of methods available to calculate LC50's (reviewed in Stephan 1977). These methods range from graphical interpretation of an eye-fitted line on log-probability graph paper to probit analysis on mainframe computer. The suitability of each method in any specific case is dependent upon the results of the toxicity test. Based upon criteria listed in Stephan (1977), we have selected the following methods as most suitable for our data.

Criteria	Test	Reference
One or more test containers with partial kills; mortality ranges from 0 to 100%.	Trimmed Spearman-Karber	Hamilton et al. (1977)
One or more test containers with partial kills; no 100% mortality.	Moving average angle method	Dryer (1985); EPS, pers. comm.
Only 0 and 100% mortality.	Binomial test	Stephan (1977)

Results are reported as 96hLC50 in ppm herbicide (active ingredient) or ppb copper within specified confidence limits. Confidence limits for the Trimmed Spearman-Karber Method and the Moving Average Angle Method are 95%. Confidence limits for the binomial test are calculated as shown below and ranged from 98.4% to 99.9%. Data for copper (Experiment 3) and ester (Experiment 5) did not fit any of the criteria in the above table and there were not enough partial mortalities to fit the criteria for probit analysis. Therefore, in these two cases we estimated the LC50 by the Moving Average Method and by eye-fitting a line on log-probit graph paper.

The binomial test is a simple but effective method by which to calculate LC50's when only 0 and 100% mortalities occur (Stephan 1977). The LC50 is

simply the square root of A and B where A=highest concentration with 0 mortality and B=lowest concentration with 100% mortality. Confidence limits are calculated by the formula $100[1-2(1/2)^n]$ where n=no. of organisms used.

No correction factors such as Abbott's Formula (APHA 1975) were applied to our mortality data to correct for control mortality. Mortality in controls was not a major problem in the present study. Of a total of 13 control containers, only five had mortalities (one fish each; Appendix 1). Percent mortality in these five ranged from 9-14%. If total number of control fish per experiment are grouped by experiment and temperature then control mortality ranged from 0-10%. Thus, rather than apply a factor that may be of questionable validity (Stephan 1977), we report actual control mortality data with our toxicity data (Appendix 1).

As described in the previous section ('Toxicant Concentrations and Correction Factors'), nominal concentration values were used for the calculations involving the amine formulation data. Corrected values were used for the determination of LC50's for copper, glyphosate and ester.

RESULTS AND DISCUSSION

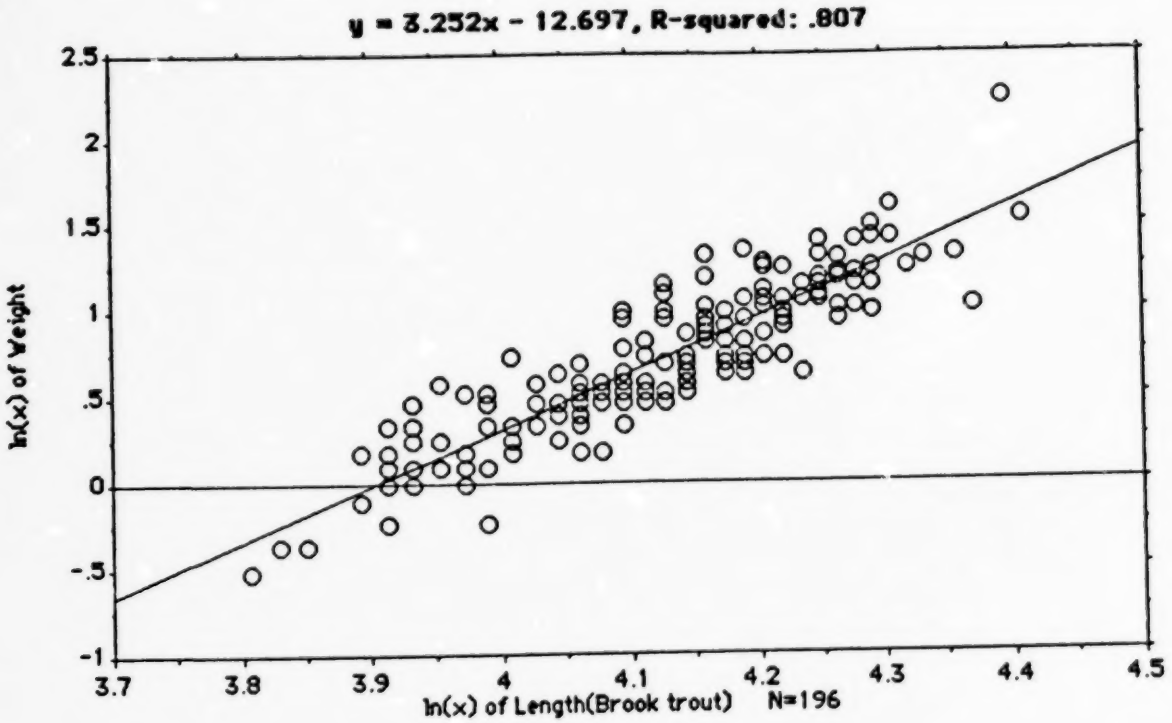
Characteristics of Test Specimens

Test specimens of brook trout ranged in size from a minimum of 45mm, 0.6g to a maximum of 82mm, 9.4g; mean size was 62mm(+/-7mm), 2.2g(+/-1.0g). Salmon ranged in size from 41mm, 0.6g to 91mm, 8.2g with a mean size of 61mm(+/-8), 2.4g(+/-1.2). Length-weight (log) regressions are shown in Figure 3. Mean fish sizes for each individual test are given in Table 3. A concerted effort was made in each stream to sample only the smallest size classes and it is assumed that all of the test specimens were YOY. However, it is possible that some of the larger specimens were older juveniles.

In general, the test specimens appeared to be hardy and fed aggressively. The trout were not quite as hardy in the holding tanks as the salmon were and they suffered some mortality. Some specific observations concerning the behaviour of test specimens in the toxicant mixtures are given in the following sections.

Environmental Conditions

Temperatures in the test containers, as measured periodically by mercury thermometers, never varied more than 1.0°C from the test temperatures of 10°, 15°, and 20°C. Oxygen concentrations in the test containers declined during the 96h unaerated tests, varying from a high of 14mg/L to a low of 6mg/L. Data were not extensive enough to greatly detail the chemical behaviour, as measured by such parameters as pH, of each test container. pH generally decreased with time for all toxicant mixtures; ranges are shown below.



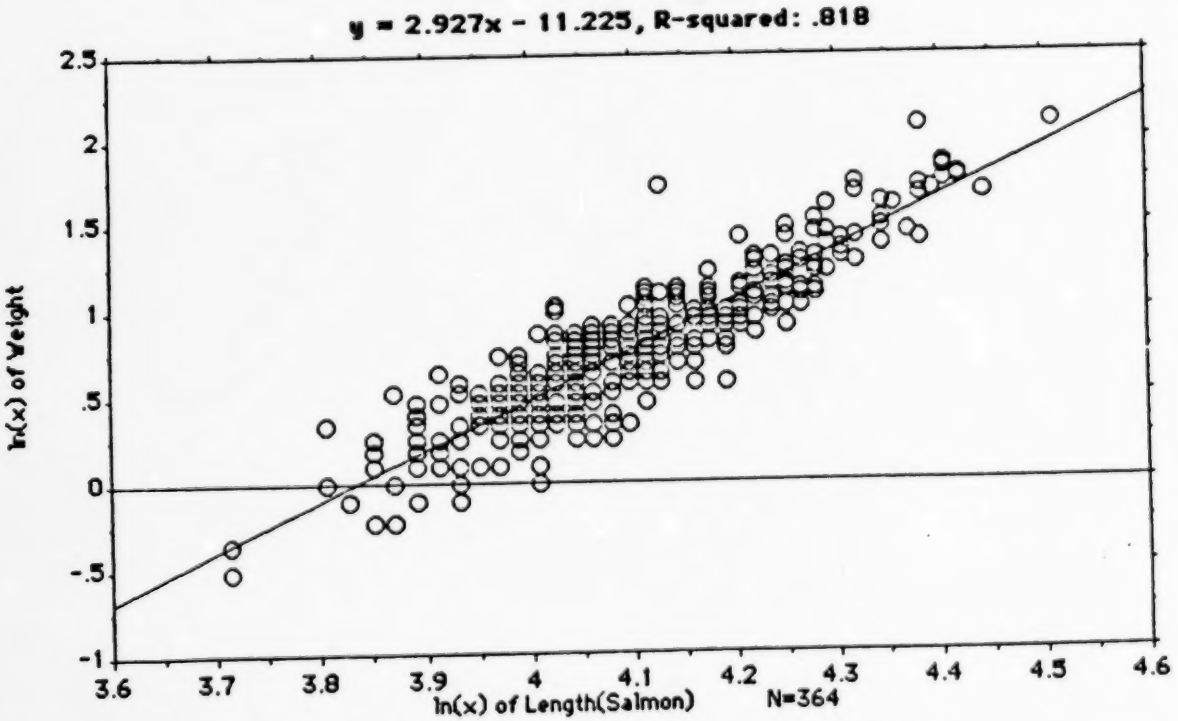


Figure 3. Length-weight regressions for test specimens of brook trout and Atlantic salmon.

Table 3. Length and weight of brook trout and Atlantic salmon test specimens used during the present study.

Experiment	Toxicant	Species	T(°C)	Length (mm)	S.D.	Weight (g)	S.D.
One	Amine	Brook	15	64	7	2.4	0.9
One	Glyphosate	Brook	15	62	7	2.3	1.0
Two	Ester	Brook	15	62	8	2.4	1.4
Three	Amine	Salmon	15	61	9	2.4	1.3
Three	Glyphosate	Salmon	15	63	9	2.6	1.2
Three	$CuCl_2$	Salmon	15	63	11	2.6	1.5
Four	Ester	Salmon	10	59	8	2.3	1.3
Four	$CuCl_2$	Salmon	10	59	8	2.2	0.9
Five	Ester	Salmon	20	58	7	2.3	0.8
Five	$CuCl_2$	Salmon	20	60	9	2.0	0.8

Toxicant	Range of pH
Controls	6.23-6.80
Amine	6.37-7.30
Ester	6.25-7.35
Glyphosate	5.84-6.38
Copper	6.39-6.97

Reference Toxicant

Cupric chloride ($CuCL_2*2H_2O$) was utilized as a reference toxicant in the present. Copper is very toxic to salmonids and is a commonly used toxicant in toxicity testing. Normally solutions are made from copper sulfate (e.g. Sprague 1964; Lauren et al. 1986) or cupric chloride (e.g. Cusimano et al. 1986); LC50 results are expressed in ppb of total copper.

The reference toxicant was used to provide some indication of the overall health and condition of our test animals. Results showed that test specimens were in good condition relative to others that have been tested. In the brook trout experiments, too little mortality occurred to calculate the LC50's. LC50's were greater than 14ppb Cu (measured) which was the highest concentration utilized. Concentrations were therefore increased for the salmon experiments. In the salmon experiments, the LC50's were 43ppb at 10°, 32ppb at 15°, and 43ppb at 20°C based on measured values of Cu (Table 4). Cusimano et al. (1986) obtained 96hLC50's for similar-sized steelhead (sea-run rainbow) trout ranging from 2.8ppb at pH 7 to 66ppb at pH 4.7 at temperatures of 14.5-17.5°C.

Toxicity of 2, 4-D Formulations

Based on LC50 results, the ester formulation (Pfizer 2,4-D Ester L.V. 600) was more toxic than the amine formulation (Pfizer dy-Amine), at least to brook trout at 15°C, with an LC50 of 31ppm for the iso-octyl ester vs. one of 125ppm for the dy-amine (Table 4). The toxicity of the ester

formulation appeared to be similar for all species and temperatures tested (31ppm for trout at 15°C; 30ppm for salmon at 10°C; 22ppm for salmon at 20°C). Both species were remarkably similar in their response to the amine formulation at 15°C (LC50's of 122ppm for salmon and 125ppm for trout) (Table 4).

There were no obvious sublethal behavioural responses to the amine formulation. Many of the trout and salmon in the ester test containers appeared to become disoriented within several hours of addition to the test containers. In addition, a number in the ester containers were observed gaping at the surface even though there was no apparent lack of oxygen.

Toxicity of Glyphosate Formulation

The glyphosate formulation (Monsanto Roundup) was, by far, the most toxic of the three formulations tested with LC50's of 2.2ppm for trout and 3.1ppm for salmon at 15°C (Table 4). Both LC50's are similar and data are probably not extensive enough to confirm a slightly higher toxicity of the formulation to brook trout compared to salmon.

Comparison with Other Studies

Few data are available from acute lethal toxicity tests that were conducted using the same species, toxicant formulations, and methods as those employed in the present study. No data are available for Newfoundland populations of brook trout and Atlantic salmon. Some comparable data that were obtained using other salmonid species are shown in Table 5.

In general , ester formulations are more toxic to fish than amine formulations (Newfoundland Forests and Lands et al. 1985; present study; Table 5). It is interesting to note that glyphosate alone is not particularly toxic to fish (38-140ppm) (Table 5). The formulation utilized in the present study (trade name Roundup) contains surfactants (Newfoundland Forests and Lands et al. 1985) that are relatively toxic to fish (2ppm) (Table 5). Previous comparable studies have found Roundup to be toxic to salmonids in the 8-48ppm range (Table 5). The literature review by the Dept. of Forest Resources and Lands et al. (1985) states that 'Roundup and its surfactants

Table 4. 96hLC50's for brook trout and salmon during the present study. LL,UL=lower and upper 95% confidence limits.

Exper.	Toxicant	Species	T(°C)	LC50	LL	UL	Method
One	Amine	BT	15	125	120	131	S-K(10%)
One	Glyphosate	BT	15	2.2	2	3	S-K(10%)
Two	Ester	BT	15	31	29	33	S-K(10%)
Three	Amine	S	15	122	113	133	S-K(12%)
Three	Glyphosate	S	15	3.1	2.5	4.2	MAA
Three	Cu	S	15	32	22	47	MAA
				28	-	-	L-P
Four	Ester	S	10	30	29	32	MAA
Four	Cu	S	10	43	35	54	S-K(10%)
Five	Ester	S	20	22	19	31	MAA
				26	-	-	L-P
Five	Cu	S	20	43	18	57	Bt

Abbreviations: S-K=Trimmed Spearman-Karber(%trimmed); Bt=Binomial MAA=moving average angle; L-P=eye-fitted log probit; Test with 99.9% confidence limits; BT=brook trout; S=salmon.

Control Mortality:
Experiment 1=0,12.5,12.5 (8.3% combined)
Experiment 2=14.3,12.5 (13.3%)
Experiment 3=0,0 (0%)
Experiment 4=0,0 (0%)
Experiment 5=9.1,9.1 (9.1%)

Table 5. Comparison of 96hLC50 values from the present study with those determined in other comparable studies.

Toxicant	T(°C)	Species	Size	LC50	Reference
Dimethyl-amine salt (2,4-D)	-	Coho	YOY	>237	Lorz et al.(1979) in Dept. Forest Resources & Lands et al.(1985)
	10	Chinook	1g	>100	Johnson & Finley(1980)
	10	RT	1.4g	>100	" " "
Alkanol-amine salt, d-isomer (2,4-D)	15	AS	2.4g	122	Present study
	15	BT	2.4g	125	Present study
Isoocytyl ester (2,4-D)	10	Cut.	0.4-0.8	>50	Woodward(1982)
	10	SS	Smlt	10-50	Meehan et al.(1974)
	10	A.Coho	Finger.	>50	" " "
	10	DV	"	10-50	" " "
	10	RT	"	10-50	" " "
	10	Chum	Fry	10-50	" " "
	10	O.coho	Finger.	>50	" " "
	15	BT	2.4g	31	Present study
	10	AS	2.3g	30	" "
	20	AS	2.3g	22	" "
Glyphosate (Roundup)	12	RT	-	8.3	Folmar et al.(1979) in Dept. Forest Resources & Lands et al.(1985)
	-	Trout	-	11	Monsanto(1982b) in Dept. Forest Resources & Lands et al.(1985)

Table 5 cont.

Toxicant	T(°C)	Species	Size	LC50	Reference
Glyphosate (Roundup)	-	RT	-	48	US Dept. Agric.(1981) in Dept. Forest Resources & Lands et al.(1985)
	15	BT	2.3g	2.2	Present study
	15	AS	2.6g	3.1	Present study
Glyphosate	12	RT	-	140	Folmar et al.(1979) in Dept. Forest Resources & Lands et al.(1985)
Glyphosate	-	RT	Fry	50	Folmar(1976) in Dept. Forest Resources & Lands et al. (1985)
Glyphosate (Technical)	-	RT	-	38	US Dept.Agric.(1981) in Dept. Forest Resources & Lands et al.(1985)
Surfactant (Roundup)	12	RT	-	2	Folmar et al.(1979) in Dept. Forest Resources & Lands et al.(1985)

Abbreviations:

AS= Atlantic salmon
BT=brook trout
Cut.=cutthroat trout
A.coho=Alaska coho
DV=Dolly varden trout
O.=Oregon coho
SS=sockeye salmon
RT=rainbow trout
Smlt=smolts
Finger.=fingerlings

are generally considered toxic to fish with LC50's of 1-10ppm under laboratory conditions'. It is likely that the surfactants accounted for the relatively low (compared to the 2,4-D formulations) LC50's obtained during the present study. The amount and type of surfactant in the test solutions was not determined during the present study.

CONCLUSIONS

Study Results

1. The 96hLC50's with 95% upper and lower confidence limits for brook trout at 15°C were:

   a. 125ppm (120,131) for Pfizer 2, 4-D (dy-Amine),

   b. 31ppm (29,33) for Pfizer 2,4-D (iso-octyl ester formulation),

   c. 2.2ppm (2.0,3.0) for Monsanto Roundup (glyphosate formulation).

2. The 96hLC50's for Atlantic salmon (primarily ouananiche) at 15°C were:

   a. 122ppm (113,133) for the amine formulation, and

   b. 3.1ppm (2.5,4.2) for the glyphosate formulation.

3. The 96hLC50's for salmon at 10° and 20°C were:

   a. 30ppm (29,32) for the ester formulation at 10°, and

   b. 22ppm (19,31) for the ester formulation at 20°C.

4. Surfactants may have been responsible for the relatively high toxicity of the glyphosate formulation.

5. Sublethal effects, such as disorientation and surface gaping may be important in assessing the impact of large amounts of 2,4-D iso-octyl ester formulation entering the aquatic environment.

6. Although the present tests were not exhaustive, temperature (10, 15, or 20°C) and species (brook trout vs. Atlantic salmon) did not appear to greatly affect the outcome of the tests. However, the ester formulation appeared to be slightly more toxic at 20°C than at the lower temperatures.

Application of Results

Care must be taken in the intrepretation of the results of the present study for impact assessment or guidance of operational spraying. 96h static toxicity tests are conservative estimators of toxicity for an operational

spray program since test specimens are exposed to greater concentrations, for greater time periods, than fish are likely to encounter in the field.

For example, Dept. Forest Resources and Lands et al. (1985) estimated that 'maximum temporary' concentrations of glyphosate and 2,4-D in the aquatic environment during an operational spray program would be 0.03 and 0.05 ppm, respectively. This scenario is based upon applications of 4.8 kg a.i. 2,4-D/ha and 2.9 kg a.i. glyphosate/ha with a 30 m width buffer, 15 cm minimum water depth, and an 8 km/h wind in the direction of the water. In addition, under normal stream conditions, fish would be exposed for a very brief time as the pulse of herbicide was rapidly mixed, diluted, adsorbed to particles and moved downstream by streamflows. Measurable levels of herbicide may persist in eddies but presumably the fish could move away from potentially dangerous levels as has been reported by Folmar (1976) for 2,4-D (in Dept. Forest Resources and Lands et al. 1985). During the present study, the earliest fish to die was one in a high level glyphosate mixture that died after 9h of exposure. It is likely that the fish have to be exposed for some period of time (i.e. hours or days rather than seconds) before suffering toxic effects.

On the other hand, it should be noted that there is some uncertainty associated with the 'margin of safety' as estimated from a 96hLC50. Norris et al. (1983) suggests that a factor of 0.1 (e.g. 0.1 x 96hLC50) be utilized to calculate acceptable concentrations for operational spraying of herbicides. He based this on discussion presented in Sprague (1971) and water quality criteria contained in National Academy of Sciences-National Academy of Engineering (1973).

Another note of caution includes the fact that the present study considered only lethal effects on young-of-the-year salmonids from exposure to various mixtures of herbicides; while these life stages are more sensitive to herbicides than adult fish they are not as sensitive as yolk sac larvae. In addition, this study did not consider sublethal effects such as slowed response time, an effect that could become lethal in a fast moving stream environment, nor did it consider any potential indirect effects on salmonids such as that caused by mortality of stream invertebrates.

Considerations for an operational spray program that have arisen out of the present study include the following:

1. The glyphosate formulation was more toxic to local salmonids than the ester formulation of 2,4-D which in turn was more toxic than the amine formulation. This information should be considered when deciding which formulation to use under certain conditions. Consideration should be given to changing or eliminating the surfactant in the glyphosate formulation since this appears to be the most toxic component of the mixture.

2. Temperature, within the range of those likely to be encountered in the field, does not appear to play a large role in the toxicity of the substances tested.

3. Although it is may be unlikely that herbicide concentrations would attain levels sufficient, over a long enough period of time, to cause mortality to salmonids in the field, except under a catastrophic series of events such as a direct spill into a water body or perhaps multiple sprayings directly over standing water, there is sufficient uncertainty to exercise caution. Thus, all reasonable mitigations should be employed to ensure that concentrations remain well below those known to be lethal to salmonids.

4. A monitoring program should be instituted to assess effectiveness of impact assessment and mitigations. The water sampling regime should include some sampling at peak runoff events as soon as possible after spraying to allow comparisons of 'maximum attainable' field concentrations with laboratory toxicity data.

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APPENDIX

Appendix 1. Toxicity data.

	Nominal Concentration (ppm/ppb)	Total No. Fish	No. Dead 0h	24h	48h	72h	96h
Experiment 1							
Amine(ppm)	30	8	0	0	1	0	0
	54	7	0	0	0	0	0
	96	8	0	0	0	0	0
	168	8	0	2	2	4	
	250	7	0	7			
Glyphosate(ppm)	3.0	8	0	0	0	0	0
	5.4	8	0	0	0	1	0
	9.6	7	0	5	0	2	
	16.9	8	0	8			
	30	8	0	8			
Control 1		8	0	0	0	0	0
Control 2		8	0	1	0	0	0
Control 3		8	0	0	0	0	1
Cupric chloride(ppb Cu)	5	7	0	0	0	0	1
	20	7	0	0	0	0	0
	50	7	0	1	0	0	0
Experiment 2							
Ester(ppm)	30	8	0	0	0	0	0
	54	7	0	0	5	1	0
	96	8	0	8			
	168	8	0	8			
	250	8	0	8			
Control 1		7	0	1	0	0	0
Control 2		8	0	0	0	1	0
Cupric chloride(ppb Cu)	5	7	0	0	0	1	0
	20	7	0	0	0	0	0
	50	7	0	0	0	0	0

Appendix 1. Toxicity data.

	Nominal Concentration (ppm/ppb)	Total No. Fish	No. Dead 0h	24h	48h	72h	96h
Experiment 3							
Amine(ppm)	50	9	0	0	0	0	0
	75	9	0	0	0	0	0
	100	9	0	0	0	0	0
	125	9	0	0	2	1	2
	150	9	0	5	3	1	
Control 1		9	0	0	0	0	0
Control 2		9	0	0	0	0	0
Cupric chloride (ppb Cu)	50	9	0	0	0	1	0
	100	9	0	2	1	0	0
	200	9	0	8	1		
Glyphosate(ppm)	2	9	0	0	0	0	0
	4	9	0	0	0	0	0
	6	9	0	0	0	0	0
	8	9	0	0	0	1	2
	10	9	0	2	3	2	0
Control 3		9	0	0	0	0	0
Control 4		9	0	0	0	0	0
Experiment 4							
Ester(ppm)	30	11	0	0	0	0	0
	40	11	0	0	0	4	0
	50	11	0	0	0	3	4
	60	11	0	0	1	3	7
	70	10	0	0	3	3	1
Control 1		11	0	0	0	0	0
Control 2		11	0	0	0	0	0
Cupric chloride(ppb Cu)	50	11	0	0	0	0	0
	100	11	0	0	1	1	0
	200	10	0	8	1	0	1

Appendix 1. Toxicity data.

	Nominal Concentration (ppm/ppb)	Total No. Fish	No. Dead 0h	24h	48h	72h	96h
Experiment 5							
Ester (ppm)	30	11	0	0	1	6	0
	40	11	0	0	8	3	0
	50	11	0	5	5	0	0
	60	11	0	11			
	70	11	0	11			
Control 1		11	0	0	0	0	1
Control 2		11	0	0	0	0	1
Cupric chloride (ppb Cu)	50	11	0	0	0	0	0
	100	11	0	0	0	0	0
	200	11	0	11			